## CLAIM + DETAILED DESCRIPTION

[Claim(s)]
[Claim 1] Interleukin 18 production accelerator which contains one sort or two sorts or more of mixtures which are the medicines which promote production of interleukin 18 and are chosen from the group which consists of macrophage colony-stimulating factors and salts of these factors permitted pharmacologically as an active ingredient.
[Claim 2] Interleukin 18 production accelerator according to claim 1 whose salts of macrophage colony-stimulating factors permitted pharmacologically are acid addition salt, a metal complex, or carboxylate salt.

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the interleukin 18 production accelerator. Furthermore, this invention relates to the medicine which promotes production of the factor which controls the onset and advance of the diabetes of Homo sapiens and a mammal, and production of the factor which controls the bone metastasis of a cancer cell in detail. Unless percentage has a notice especially in this Description, it is the display by weight.
[0002]
[Description of the Prior Art] Generally specific proliferation and a differentiator are needed for proliferation of a hematopoietic cell, and differentiation, and, in between [ until it becomes various kinds of hemocyte which finally ripened, for example, red blood cells, the granulocyte, a macrophage the eosinophilic leukocyte platelet, and a lymphocyte ], much differentiation and a growth factor are involving. On the other hand, although a living body corresponds by immunoreaction to infection of bacteria, a virus, etc., a tumor, cell injury, etc., the response of the immunity is adjusted by the direct or indirect interaction between immunocompetent cells.

The biological response modulator which has the effect of acting on an immune function and raising the defense power over a foreign body attracts attention from the relation of cancer treatment in recent years. It is shown clearly one after another that the substance named cytokine, such as interleukin, colony stimulating factors, TNF(s) (tumor necrosis factor), and interferon, generically is participating in these differentiation, proliferation, and an immune function. The procedure of manufacturing cytokine with the gene engineering procedure and using as a medicine is also studied [science (Science), the 235th volume, the 1504-1508th page, 1987 and the same magazine, the 378th volume, No. 6552, the 88-91st page, and 1994].

[0003] Although 18 kinds are checked as cytokine belonging to interleukin now, interleukin 18 (it may be hereafter written as "IL-18") is a kind of the cytokine which is a signal transduction substance in an immune system in it. This cytokine is JP,H8-27189,A in the time of discovery, Although expressed as an interferon gamma inducing factor from JP,H8-191098,A, JP,H9-289896,A, the report in [Nature (Nature), the 378th volume, No. 6552, the 88-91st page, and 1994], etc. It came to be called IL-18 after that [journal OBU immunology (Journalof Immunology), the 156th volume, the 4274-4279th page, and 1996].

[0004] IL-18 are functionally [ as the interleukin 12 which is the protein of an interleukin 1 family ] similar structurally. Although IL-18 guide powerfully live birth of interferon gamma from a T cell and natural killer cells, In addition, the granulocyte and a macrophage colony-stimulating factor, a tumor necrosis factor, It has the character to guide interleukin 4, interleukin 5, interleukin 8, interleukin 10, and interleukin 13. Furthermore, [macrophage journal (Macrophage Journal) which also has the character which enhances the cell injury nature of natural killer cells, and the character to guide generation of natural killer cells, Kohei Miyazono and 6th volume, No. 3, 5-6th page, 1999, and Kazuo Sugamura work, "revised edition cytokine and a growth factor", the 46-47th page, Yodosha, and 1998 --] .

[0005] It is strongly suggested by the experiment which used the animal model that IL-18 are participating in symptoms formation of a disease. That is, it is although the manifestation of IL-18 is accepted in parallel with the activity situation of symptoms in the NOD (nonobese diabetic) mouse which is the model of insulin dependent diabetes mellitus, If IL-18 are prescribed for the patient, it will have become clear that the onset of a disease is controlled [journal OBU immunology (Journal of Immunology), the 163rd volume, the 1230-1236th page, and 1999].

[0006] Furthermore, it is reported that IL-18 have an inhibitory effect to the bone metastasis of a Homo sapiens lung cancer cell (MDA-231) etc. [anti cancer research (AnticancerResearch), the 19th volume, the 4131-4138th page, and 1999]. Moreover, it is also indicated that IL-18 have the osteoclastogenesis inhibition effect (JP,H10-236974,A). On the other hand, a macrophage colony-stimulating factor is a factor which acts on marrow cells and promotes the

differentiation to the granulocyte and a macrophage, and proliferation. The macrophage colony-stimulating factor which promotes differentiation of monocyte and a macrophage, and proliferation, The granulocyte colony-stimulating factor which promotes differentiation and proliferation of the granulocyte, the granulocyte, and differentiation of a macrophage, It is classified into the granulocyte macrophage colony-stimulating factor which promotes proliferation (Kohei Miyazono and Kazuo Sugamura work, "revised seal cytokine and a growth factor", the 46-47th page, Yodosha, 1998).

[0007] Macrophage colony-stimulating factor (Macrophage Colony Stimulating Factor.) It may be hereafter written as M-CSF. Takaku and others is the gay dimer glycoprotein of molecular weight 84,000 Dalton which discovered out of Homo sapiens urine in 1978. Act on the monocyte committed stem cell in the marrow of a mammal, and Monocyte, the differentiation to a macrophage, Promote proliferation, and act on the macrophage which ripened further and production of a granulocyte colony-stimulating factor (GranulocyteColony Stimulating Factor) is promoted. Promoting the increase in the granulocyte count in the peripheral blood which decreased in number with cancer chemotherapy etc. is known [the international conference report on mucosa immunology, the 109th page, and 1990]. Moreover, in in the living body, a macrophage colony-stimulating factor participates in formation of an osteoclast, and its functional manifestation, and has very important mechanisms, such as offer of the pulp chamber which is the place of an osteoplasty and hemopoiesis. Furthermore, since it also has the activity which accelerates mechanisms, such as lipometabolism, the adaptation as a blood cholesterol fall agent is also considered (Toshio Suda work, "the fate of a blood stem cell", the 64th page, Yodosha, 1992).

[0008] In addition, it is a use of a macrophage colony-stimulating factor, Effective (JP,S62-27010,A) in the bone marrow transplantation in a hematopoietic forming organ disease, The antitumor effect of a cancer immunity treatment agent is made to increase (JP,H1-193227,A), Effective (JP,H1-207244,A) in prevention of a decrease of platelets, Effective (JP,H6-10137,B) in functional improvement of transplanted bone marrow, Effective (JP,H2-225418,A) in the treatment of a malignant tumor, Effective (JP,H2-264729,A) as an auxiliary material of a platinum complex anticancer drug, effective (JP,H2-258728,A --) in the treatment of hyperlipemia Effective (JP,H3-17021,A) in the treatment of JP,H2-264728,A and JP,H3-2125,A, and myelodysplastic syndromes, It is indicated that it is effective (JP,H8-104648,A) in prevention and treatment of a nephritis and effective in the treatment of the osteopetrosis (JP,H4-178334,A) etc. However, it is not known that these macrophage colony-stimulating factors have an IL-18 production facilitatory effect, and it is not indicated in literature, either.

[0009]

[Problem(s) to be Solved by the Invention] As a result of inquiring wholeheartedly about the substance which promotes production of the factor which controls the diabetic onset and

advance, and the factor which controls the bone metastasis of a cancer cell in view of said conventional technology, this invention persons found out that the operation concerned existed in macrophage colony-stimulating factors, and completed this invention. This invention aims at offering the production accelerator of the factor which controls the diabetic onset and advance, and the factor which controls the bone metastasis of a cancer cell.

[0010]

[Means for Solving the Problem] This invention which solves said technical problem is a medicine which promotes production of interleukin 18. It is the interleukin 18 production accelerator which contains one sort or two sorts or more of mixtures chosen from the group which consists of macrophage colony-stimulating factors and salts of these factors permitted pharmacologically as an active ingredient. It requires also as a desirable mode that the salts of macrophage colony-stimulating factors permitted pharmacologically are the interleukin 18 production accelerator which is the macrophage colony-stimulating factor which is acid addition salt, a metal complex, or carboxylate salt.

[0011]

[Embodiment of the Invention] Next, this invention is explained in detail. The active ingredients which promote the production of a factor which has the operation which controls the diabetic onset and the advance in this invention, and the operation which controls the bone metastasis of a cancer cell are one sort or two sorts or more of mixtures chosen from the group which consists of macrophage colony-stimulating factors and salts of these factors permitted pharmacologically. The macrophage colony-stimulating factors used for this invention are the derivatives permitted like pharmacology of a macrophage colony-stimulating factor or a macrophage colony-stimulating factor, and it is the macrophage colony-stimulating factor itself especially desirably. Although not restricted especially as a manufacturing process of a macrophage colony-stimulating factor, it can also manufacture in gene engineering possible [ also isolating by the physicochemical procedure out of Homo sapiens urine ], for example.

[0012] It will be as follows if a concrete manufacturing process is illustrated. As a procedure chemically isolated from Homo sapiens urine, JP,S63-198700,A, The procedure indicated by JP,H1-502397,A etc. as procedures of manufacturing in gene engineering, such as JP,S63-250400,A, JP,S64-22899,A, and JP,S62-501607,A, is known. The macrophage colony-stimulating factor manufactured by the aforementioned procedure acts on the marrow cells of a mammal in soft agar, and has the activity (it may be indicated as colony stimulating activity below) which promotes the colony formation which consists of monocyte and a macrophage system cell.

[0013] If it is the factor which has the same colony stimulating activity as this as macrophage colony-stimulating factors used in this invention, it will be altogether usable and the kind in particular will not be limited. Especially the macrophage colony-stimulating factor

manufactured by the manufacturing process of JP,H1-502397,A is more specifically desirable. The peptide which has the amino acid sequence of the macrophage colony-stimulating factor concerned, Although the peptide which a part of amino acid sequence of the macrophage colony-stimulating factor concerned dropped out or replaced, the peptide which other amino acids inserted or added to a part of amino acid sequence of the macrophage colony-stimulating factor concerned, etc. can be illustrated It is not limited to these, but if it has said colony stimulating activity, all can be used for this invention.

[0014] [ the macrophage colony-stimulating factor obtained by the chemical procedure or the gene engineering procedure as aforementioned ] The buffer solution which contains a human serum albumin, the sugars, etc. as stabilizer as occasion demands can be added, a macrophage colony-stimulating factor can be dissolved, hereafter, sterile filtration is carried out by a usual state method, it can freeze-dry and the IL-18 production accelerator can be manufactured. In addition, it is also possible to add the component permitted in pharmacology besides a human serum albumin and the sugars.

[0015] [ the active ingredient which promotes production of IL-18 in this invention ] It is one sort or two sorts or more of mixtures chosen from the group which consists of said macrophage colony-stimulating factors and salts of these factors permitted pharmacologically, and avirulent salt, such as acid addition salt and a metal complex, carboxylate salt, etc. can be illustrated as salts of these factors permitted pharmacologically. As acid addition salt, more specifically The hydrochloride, sulfate, phosphate, the oxalate, As a metal complex, acetate, citrate, ascorbate, the tartrate, etc. Zinc, Complexes, such as iron, calcium, magnesium, and aluminum, can be illustrated for alkaline-earth-metals salt, such as alkali metal salt, such as sodium salt and potassium salt, calcium salt, and magnesium salt, ammonium salt, etc. as carboxylate salt. These salts can be manufactured by a usual state method.

[0016] [ the IL-18 production accelerator of this invention / the salts of the macrophage colony-stimulating factors manufactured as aforementioned, and/or macrophage colony-stimulating factors permitted pharmacologically ] For example, it is possible to sterilize by a usual state method, to dissolve in the physiological saline for injection, the water for injection, etc., and to manufacture a tablet. It can dissolve in the physiological saline for injection, the water for injection, etc., and the IL-18 production accelerator of this invention can prescribe the effective dose for the patient suitably again according to age, symptoms, etc. according to proper forms, such as intravenous drip, intravenous injection, a subcutaneous injection, and intraperitoneal injection, for example.

[0017] Since the IL-18 production accelerator of this invention is using as the active ingredient the macrophage colony-stimulating factor which is the product of nature originally included automatically in human body fluid, there is an advantage that side effects are very slight. In intravenous administration, as for LD50 of the result of an animal examination to this these

these macrophage colony-stimulating factor, more than 300mg [/kg ] weight is [ more than 100mg //kg / weight / at internal use ] more than 200mg [/kg ] weight in hypodermic administration. The effective dose of the IL-18 production accelerator of this invention is converted from the animal test result which carries out a postscript, and is about 500mug/day [/body ] pile kg.

[0018] Next, the example of an examination is shown and the operation effect of this invention is explained concretely.

the example 1 of an examination -- this examination carries out a fixed quantity of IL-18 guided into the spleen of the mouse which prescribed the macrophage colony-stimulating factor for the patient -- it went to accumulate.

[0019] (1) 60 five weeks of treatment **** C57BL/6 mice (from Japanese CHARUSURIBA to purchase) of preparation 1 test animal of a sample were acclimated for one week by the usual state method, the back divided into six groups of one groups [ ten ] at random, and each group was processed as follows.

** 200micro of placebo group physiological saline (made by Otsuka Pharmaceutical) l / mouse was prescribed for the patient from the caudal vein.

** About medication group Homo sapiens M-CSF(made by Morinaga Milk Industry Co., Ltd.) 500microg/body pile kg, it is 1 time (for one day.). A 1-time medication group, 3 times (for three days.) A 3 times medication group, 5 times (for five days.) A 5 times medication group, 7 times (for seven days.) A 7 times medication group, 9 times (for nine days.) A medicine was prescribed for the patient from the medication group caudal vein 9 times, and the spleen was extracted 3 hours after the last medication.

[0020] 2) Put the spleen in which the spleen carried out treatment extraction into a hand homogenizer. [ 1ml per 100mg of organs of homogenization solution [9.1mM hydrogen-phosphate disodium (made by a Wako Pure Chem industrial company), 1.7mM phosphoric-acid 2 hydrogen sodium (made by a Wako Pure Chem industrial company), and 150mM sodium chloride (made by a Wako Pure Chem industrial company) ] Phosphate buffer solution pH7.4 included and 1% of last concentration NP 40 (made by a sigma company), And the 0.5% of last concentration sodium deoxycholate (made by a Wako Pure Chem industrial company), And solution] containing the 0.1% of last concentration sodium dodecyl sulfate (made by a Wako Pure Chem industrial company) and the last 100microg (p-amidino phenyl)/ml concentration methanesulfonyl fluoride hydrochloride (made by a Wako Pure Chem industrial company) was added, and it uniformed. Then, the whole quantity was moved to the micro tube, at-long-intervals heart separation was carried out by 4 degrees C and 15,000 rotations for 10 minutes, supernatants were collected, centrifugal separation was further carried out on these conditions, supernatants were collected, and the sample was prepared.

[0021] (2) 100micro of test method profit **** each sample g -- sodium-dodecyl-sulfate (it is

hereafter written as SDS.) polyacrylamide electrophoresis gel (made by TEFUKO.) SDS electrophoresis was performed using 16% of concentration. It is a polyvinylidene difluoride membrane filter (made by Amersham Pharmacia Biotech K.K.) about the protein separated after the end of electrophoresis, and into gel. Hybond-P. A catalog number, RPN2020F. 10mM tris hydrochloric acid buffer solution pH7.4 which transfer and contain skim milk (made in Difco Laboratories) 5% (made by a Wako Pure Chem industrial company), Blocking was performed at room temperature for 2 hours using 150mM sodium chloride (made by a Wako Pure Chem industrial company), and the solution which contains TSUIN 20 (made by a sigma company) 0.1%. Subsequently, 10mM tris hydrochloric acid buffer solution pH7.4 which contain skim milk 0.5%, 150mM sodium chloride, [ with and the solution (it is hereafter written as the antibody dilution.) which contains TSUIN 20 and 0.2% sodium azide (made by Nakarai Tesuku) 0.1% ] The anti-mouse IL-18 goat antibody (made by R and D systems company) solution diluted to the 0.1microg/ml last concentration was made into the primary antibody, and was made to react one nights at 4 degrees C.

[0022] After [ the end of an after-reaction ], and 10mM tris hydrochloric acid buffer solution pH7.4, 150mM sodium chloride, And it washed by TSUIN 20 (it is hereafter written as the wash.) 0.1%, and was made to react at room temperature for 1 hour by making into a second antibody the horseradish peroxidase marker-anti-goat IgG-rabbit antibody (made by eye See N) further diluted with the antibody dilution 10,000 times. The wash washes a membrane filter after the end of a reaction, and it dips in the reaction mixture for ECL-Plus Western-blotting detection (made by Amersham Pharmacia Biotech K.K.) for 5 minutes. The band which the hyper-film ECL (made by Amersham Pharmacia Biotech K.K.) was made to expose, and was exposed was made a fixed quantity with the densitometer, and the average value of each group was computed and examined.

[0023] (3) an examination result -- the result of this examination is as being shown in Table 1. Table 1 shows the result of having made a fixed quantity the band of IL-18 detected by the western blotting method in DENTOSHITO meter. A passage clear from Table 1 A placebo group, an one Homo sapiens M-CSF medication group, [ group / a three Homo sapiens M-CSF medication group, a five Homo sapiens M-CSF medication group, a seven Homo sapiens M-CSF medication group, and / nine Homo sapiens M-CSF medication / the amount of manifestations of IL-18 in the extracted spleen ] It is 64ng, 85ng, 302ng, 753ng, 1291ng, and 1075ng, respectively, and the production amount of IL-18 of a seven Homo sapiens M-CSF medication group showed maximum. Therefore, it became clear that it was required to prescribe Homo sapiens M-CSF for the patient at least 7 times. In addition, although examined by changing the kind of M-CSF, the almost same result was obtained.

[0024]

[Table 1]

| ヒトM－CSF投与群 | 脾臓のIL－18産生量（ng） |
|---|---|
| プラセボ群 | 64 |
| 1回投与群 | 85 |
| 3回投与群 | 302 |
| 5回投与群 | 753 |
| 7回投与群 | 1，291 |
| 9回投与群 | 1，075 |

[0025] the example 2 of an examination -- this examination examines the acute toxicity of M-CSF which is the main ingredients of the medicine of this invention -- it went to accumulate.
(1) It dissolved in the physiological saline for injection (made by Otsuka Pharmaceutical), sterile filtration of preparation Homo sapiens M-CSF (made by Morinaga Milk Industry Co., Ltd.) of a sample was carried out, and the sample solution was prepared.
[0026] (2) The test method acute toxicity test method was performed in [a fundamental, clinical, the 22nd volume, No. 7, the 1649-1659th page, and 1988] using the 8-weeks old mouse (the inside of a vein, hypodermic, taking orally) based on the test method of a description.
[0027] (3) a test result -- there is no example of death also in which medication group -- pathology -- histologically, change was not accepted. Within the vein, as for LD50 of the Homo sapiens M-CSF concerned, more than 300mg [/kg ] weight was [ more than 100mg //kg / weight ] more than 200mg [/kg ] weight in internal use at hypodermic administration. Next, although a work example is shown and this invention is explained still in detail, this invention is not limited to the following work examples.
[0028]
[Example] The injection of composition of the primary work example was manufactured by the usual state method.
Homo sapiens M-CSF (made by Morinaga Milk Industry Co., Ltd.) 0.5 (%)
Sodium chloride (made by Wako Pure Chem) 0.9 Water for injection (made by Otsuka Pharmaceutical) 98.6 [0029] The injection of composition of the secondary work example was manufactured by the usual state method.
Homo sapiens M-CSF (made by Morinaga Milk Industry Co., Ltd.) 0.5 (%)
Actinomycin-D (made by a sigma company) 0.005 Sodium chloride (made by a Wako Pure Chem industrial company) 0.9 Water for injection (made by Otsuka Pharmaceutical) 98.595
[0030] The tablet of the following composition was manufactured by the usual state method per 31 doses of work examples.
Homo sapiens M-CSF (made by Morinaga Milk Industry Co., Ltd.) 1.0 (mg)
Actinomycin-D (made by a sigma company) 0.02 Lactose (made by a Wako Pure Chem industrial company) 162.98 Crystalline cellulose (made by a Wako Pure Chem industrial company) 30.0 Polyvinylpyrrolidone (made by a sigma company) 5.0 [0031]

[Effect of the Invention] The effect of this invention relating to the new IL-18 production accelerator, and being done so by this invention is as follows as indicated in details above.
1) Production of IL-18 can be promoted certainly in the living body of Homo sapiens and a mammal.
2) M-CSF is already approved as a drug, the safety is checked, and the side effects by medication of the tablet of this invention are not accepted.
3) The IL-18 production accelerator of this invention is effective in the effect which controls the diabetic onset and advance, and bone metastasis suppression of a cancer cell.

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[Translation done.]